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# Effect of Calcium on In Vitro Activity of LY146032 against *Clostridium difficile*

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**The in vitro MICs of LY146032 against 63 isolates of *Clostridium difficile* tested in Wilkins-Chalgren broth ranged from 0.5 to >32 μg/ml, with MICs of 4 and 8 μg/ml for 50 and 90% of the isolates, respectively. However, when the test medium was supplemented with physiologic concentrations of calcium, the MIC for 90% of the isolates was reduced to ≤0.12 μg/ml.**

LY146032 is a biosynthetic antibacterial agent which belongs to a new class of antibiotics known as peptolides (acidic lipopeptide antibiotics). LY146032 is synthesized by *n*-decanoyl acylation of the terminal amino group of a deacylated cyclic polypeptide antibiotic, A21978$C_1$, derived from *Streptomyces roseosporus* (1). The peptolides LY146032 and A21978$C_1$ differ only in their fatty acid acyl groups and have good inhibitory and lethal activities against a broad range of gram-positive but not gram-negative bacteria. LY146032 inhibits an early step in cell wall biosynthesis before the formation of UDP-*N*-acetyl-muramyl-pentapeptide. Additionally, there is evidence that LY146032 may also have an effect on the cytoplasmic membrane of bacteria because L forms were inhibited by this agent and selective permeability changes were observed in organisms exposed to LY146032 (N. Allen, W. Alborn, J. Hobbs, and H. Percifield, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1081, 1984). Preliminary studies of the pharmacokinetic properties of LY146032 indicate a serum half-life of 2.2 h in rats, relatively low toxicity to animals, and effectiveness in treating experimental endocarditis and pyelonephritis in rats. (A. M. F. Hansen, D. H. Holmes, D. A. Preston, and R. S. Pekarek, 24th ICAAC, abstr. no. 1079, 1984).

Because the preliminary reports of the in vitro activity, pharmacokinetic properties, and in vivo response to LY146032 are favorable, we extended the in vitro analysis of this drug by comparing its activity against *Clostridium difficile* with that of other gram-positive-bacteria-directed antibacterial agents.

The 63 strains of *C. difficile* tested in this study were clinical isolates collected at Barnes Hospital. Organisms were identified by standard methodology and stored at −70°C in skim milk. Pharmaceutical-grade powders of LY146032 (Eli Lilly & Co., Indianapolis, Ind.), vancomycin (Eli Lilly & Co.), and teicoplanin (Merrell Dow Pharmaceuticals Inc., Cincinnati, Ohio) were obtained from the manufacturers.

The MICs of the antibiotics against *C. difficile* were determined by a broth macrodilution procedure performed in accordance with the recommendations of the National Committee for Clinical Laboratory Standards (2). Unsupplemented Wilkins-Chalgren broth (3) and broth supplemented with calcium (50 μg/ml) and magnesium (25 μg/ml) were used in the susceptibility tests. An inoculum of $10^5$ CFU/ml was used, and tubes were incubated under anaerobic conditions at 35°C for 48 h.

The in vitro activity of LY146032 against *C. difficile* isolates in unsupplemented Wilkins-Chalgren medium was appreciably lower than those of vancomycin and teicoplanin (Table 1). The MICs of LY146032 against *C. difficile* ranged from 0.5 to >32 μg/ml, whereas the MICs of vancomycin and teicoplanin did not exceed 0.5 μg/ml. In addition, the MICs of LY146032 for 50 and 90% of the *C. difficile* isolates were 16- to 64-fold higher than those of vancomycin or teicoplanin. When Wilkins-Chalgren broth was supplemented with physiologic concentrations of calcium and magnesium, the MICs of vancomycin and teicoplanin against *C. difficile* remained unchanged, whereas the LY146032 MICs dropped to levels comparable to the teicoplanin MICs (≤0.12 μg/ml). The reduction in MICs was a result of the presence of physiologic concentrations of calcium and not magnesium (Table 2). It has been shown that LY146032 and the other peptolide, A21978$C_1$, have greatly diminished activity in the absence of calcium ions (1; Allen et al., 24th ICAAC). The basis for the dependence of peptolides on the presence of calcium for their activity has not been delineated. Some possible explanations include the need for cations to help in penetration of the agent into the bacterial cell or the need for calcium in the direct antimicrobial effect on peptidoglycan synthesis. It is also possible that following some critical cytoplasmic membrane changes caused by LY146032, excess calcium accumulates intracellularly, resulting in damage to bacterial cells (1).

Regardless of the basis for the observed calcium effect, it is clear that anaerobic susceptibility tests performed as currently recommended would produce misleadingly high MICs for *C. difficile*. Supplementation of Wilkins-Chalgren

TABLE 1. In vitro activity of LY146032 against 63 isolates of *C. difficile*

| Antibiotic[a] | MIC (μg/ml)[b] | | |
|---|---|---|---|
| | Range | 50% | 90% |
| LY146032 (unsupplemented broth) | 0.5–>32 | 4 | 8 |
| LY146032 (supplemented broth) | ≤0.12–0.5 | ≤0.12 | ≤0.12 |
| Vancomycin | ≤0.12–0.5 | 0.25 | 0.5 |
| Teicoplanin | ≤0.12–0.25 | ≤0.12 | ≤0.12 |

[a] Susceptibility tests were performed with Wilkins-Chalgren anaerobic broth. Supplemented broth contained calcium (50 μg/ml) and magnesium (25 μg/ml).
[b] 50% and 90%, MIC for 50 and 90% of the isolates, respectively.

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TABLE 2. Effect of calcium and magnesium on the in vitro activity of LY146032

| *C. difficile* strain | MIC (μg/ml) of LY146032 in broth with: | | | |
|---|---|---|---|---|
| | $Ca^{2+}$ and $Mg^{2+}$ | $Ca^{2+}$ only | $Mg^{2+}$ only | No supplement |
| 8534 | ≤0.12 | ≤0.12 | 4 | 4 |
| 15108 | ≤0.12 | ≤0.12 | 4 | 4 |
| 70609 | ≤0.12 | ≤0.12 | 2 | 2 |

medium with physiologic concentrations of calcium would seem appropriate, although this will have to be confirmed by in vivo studies. Given its low toxicity in animal trials and its effectiveness against problematic organisms (e.g., *Enterococcus* spp. and oxacillin-resistant *Staphylococcus* spp.), LY146032 warrants further investigation with clinical trials to confirm these findings.

## LITERATURE CITED

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